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Molecular Plasticity of Adult Bergmann Fibers Is Associated with Radial Migration of Grafted Purkinje Cells

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Embryonic Purkinje cells (PCs) from cerebellar primordia grafted in adult *pcd* mutant cerebellum replace missing PCs of th host, and become synaptically integrated into the de f ctive cerebellar circuit. This process of neuronal replacement starts with the invasion of grafted PCs into the host cerebellum, and their radial migration through its molecular lay r. Th present study is aimed at determining whether th glial axes for this migration are embryonic radial glial ells that comigrate with the grafted PCs, or adult Bergmann fibers of the host, transiently reexpressing the molecular cu s needed for their guidance of the migration. Transplants from a transgenic mouse line (Krox-20/lacZ14) in which Bergmann fibers could be identified by lacZ expression reveal that, d spite the presence of X-gal–stained Bergmann fibers in the graft remnants and of grafted PCs in the host molecular layer, all Bergmann fibers in the host cerebellum lack of β-galactosidase activity. Thus, these migratory axes belong to th host, not to the donor. Transplants from normal isog nic mouse embryos show that during the radial migration f grafted PCs (7 d after grafting) the involved host Bergmann fibers reexpress nestin (identified with monoclonal antibody Rat-401 immunostaining), normally expressed only by immature Bergmann fibers. Five days later, when grafted PCs have arrested their migration, host Bergmann fibers again become Rat-401 negative. These results indicate that mbryonic PCs can trigger in adult cerebellum the molecular changes necessary for their own migration and ultimate synaptic integration in the host cortical circuitry.

[Key words: Purkinje cell migration, embryonic and adult neural cell interactions, cerebellar grafting, nestin expression in adult Bergmann fibers, molecular plasticity, glial axes for neuronal migration]

Neurons in adult mouse cerebellum do not proliferate. In consequence, degenerating neurons cannot be replaced by a self-repair process. Embryonic neurons, however, can be grafted into adult cerebellum and become functionally integrated into the synaptic circuitry of the cerebellar cortex of the host (Sotelo and Alvarado-Mallart, 1991). The cerebellar mutant mouse strains *pcd*, *nr*, and *Lc*, in which Purkinje cells (PCs) die (Sidman and Green, 1970; Mullen et al., 1976; Caddy and Biscoe, 1979; Wassef et al., 1987; Dumesnil-Bousez and Sotelo, 1992), have provided models for studying graft integration (Sotelo and Alvarado-Mallart, 1986, 1987a, 1991, 1992; Dumesnil-Bousez and Sotelo, 1993). Penetration of grafted PCs into the cerebellum of mutant hosts begins 3–4 d after grafting (DAG 3–4) by tangential migration at the surface of the folia adjacent to the graft, followed by radial migration into the folia along Bergmann glial fibers (DAG 5–8). By DAG 10 all leading processes of PCs have arrived at the border of the host molecular and granule layer, provoking a stop signal for the migration (Sotelo and Alvarado-Mallart, 1987b; Sotelo et al., 1990).

The timing and the nature of the cellular interactions involved in the migration of grafted PCs into the host cerebellum are similar to those that take place during normal ontogeny, suggesting that, in both cases, the developmental program is regulated autonomously by the participating cells, independent of external factors, as by an internal clock (Sotelo and Alvarado-Mallart, 1987b; Sotelo et al., 1990). Although little is known about the molecular identity of the signals involved, it is clear that they are developmentally regulated and transiently expressed. During ontogenesis, it might be thought that expression of the necessary signals by the participating cells is coordinated because the cells involved are similar in age. This would not be the case, however, when embryonic neurons are grafted into adult brain. This raises the question as to whether the grafted embryonic PCs induce adult host cells, in particular the Bergmann glia, to transiently reexpress the molecular signals needed for their migration and synaptic integration into the host (Sotelo and Alvarado-Mallart, 1987b), or whether isochronic embryonic astrocytes, which express the appropriate factors because of their age, also migrate from the graft, acquire the Bergmann phenotype, and provide the substrate for migration of the PCs.

In order to determine whether comigration of embryonic PCs and astrocytes, or "rejuvenation" of host glia by the embryonic PCs is involved in graft integration, the following experiment was performed. Adult *pcd* mutant mice, in which PCs die between postnatal days 17 and 45 (Mullen et al., 1976), were grafted with cerebellar primordia from homozygous embryos of a transgenic mouse line, Krox-20/lacZ14, which shows an ectopic expression pattern of the hybrid Krox-20/lacZ gene (M. Frain, P. Charnay, R. Werhle, and C. Sotelo, unpublished observations). In transgenic animals, β-galactosidase activity

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detected in the cerebellum, exclusively in Golgi epithelial cells and their Bergmann fibers. Matured Bergmann fibers originating from the transgenic graft could, therefore, be distinguished from Bergmann fibers of the *pcd* host.

In order to determine whether, during the migration of grafted PCs, Bergmann fibers of the adult host express antigens corresponding to an earlier developmental stage, sections of cerebella that had received transplants from isogenic mouse embryos were immunostained during tangential migration (DAG 5), during radial migration on the Bergmann fibers (DAG 7), and after migration is completed (DAG 13), with the monoclonal antibody (mAb) Rat-401. This antibody recognizes nestin, an antigen expressed in proliferating cells during neurogenesis (Lendahl et al., 1990), and also transiently stains the glial axes guiding neuronal migration in the developing CNS of the rat (Hockfield and McKay, 1985).

We report (1) that transplanted cells expressing the lacZ gene do not migrate from the graft to the host molecular layer, and (2) that there is a close spatiotemporal correlation between expression of the Rat-401 antigen by host Bergmann glial fibers and radial migration of grafted PCs. It is concluded that grafted embryonic PCs induce, in the cerebellum of adult PC-deficient mice, transient expression of signals associated with their migration that recapitulate mechanisms employed in normal ontogenesis.

Materials and Methods

Mutant mice. The *pcd* mutant mice were obtained primarily from the Jackson Laboratory (Bar Harbor, ME) and raised at the Pasteur Institute (Unité Génétique des Mammifères, Paris). Homozygous *pcd/pcd* were obtained by intercrossing heterozygous mice.

Hybrid gene construction and generation of transgenic mice. Two BglII fragments of 7 and 7.2 kilobases (kb), respectively, were recovered from the 16/13 cosmid (Chavrier et al. 1989) containing the mouse Krox-20 gene and cloned into the BamHI site of pBluescript KS to reconstruct the Krox-20 gene. A 3 kb BamHI fragment from plasmid pMC1871 (Pharmacia), containing the *Escherichia coli* lacZ gene deleted of the first eight codons, was then introduced into the unique internal BglII site to generate a Krox-20/lacZ fusion gene. For egg microinjection, an 11 kb SacII-SpeI fragment, which includes the Krox-20/lacZ hybrid gene, ~2 kb of 5′ flanking sequences, and ~1 kb of 3′ flanking sequences, was excised. Linear DNA was isolated by electrophoresis on agarose gel, purified on Elutip D (Schleicher and Schüll), and injected into zygotes of B6D2 F1 females mated to identical males. Production of transgenic mice was carried out as described by Hogan et al. (1986). Transgenic mice carrying the Krox-20/lacZ hybrid gene were identified by tail DNA analysis and bred to B6D F1 males to establish transgenic lines. Expression of the transgene was analyzed by staining the embryos for β-galactosidase activity (Sanes et al. 1986).

Transplantation into adult pcd *cerebellum.* Donor tissue was obtained from the cerebellar primordia of 12 d (E12) Krox-20/lacZ14 homozygous transgenic embryos or C57BL/6J embryos (mating day was considered E0). The embryos were removed individually from the mother under chloral hydrate anesthesia (300 mg/kg body weight) and placed into phosphate-buffered saline (pH 7.2) with glucose (6 mg/ml), for the duration of the grafting procedure. Overlying tissue layers and pia were removed and the cerebellar primordia were dissected out, with microdissecting tweezers under an operating microscope. Only central portions of the metencephalic alar plates were used for transplantation to avoid grafting noncerebellar neuroepithelium. The tissue was cut into small blocks of about 0.5–1 mm diameter, which served as solid grafts. Hosts were homozygous *pcd* mutant mice of the C57BL/cdj strain, aged 2–6 months. They were anesthetized with chloral hydrate, and immobilized in a David Kopf stereotaxic frame adapted for small rodents. After craniotomy of the occipital bone, the dorsal surface of the posterior vermal lobe was exposed. Individual pieces of the selected embryonic cerebellar tissue were drawn into a glass cannula connected by a plastic tube to a 10 μl Hamilton syringe. The tip of the micropipette was inserted by hand in a posteroanterior direction, and the graft was deposited at variable depths within the host cerebellar parenchyma. After grafting, the skin was closed, and the mouse was warmed and returned to its cage. Mice were housed two or three to a cage with free access to food and water, and a 12 hr:12 hr day/night schedule. Mutant mice implanted with Krox-20/lacZ14 primordia survived for 1–2 months; those implanted with isogenic embryos survived 5, 7, and 13 d.

Perfusion. Mice carrying the Krox-20/lacZ14 transgene (transgenic mice, and *pcd* mice transplanted with donor tissue from transgenic embryos) were fixed intracardially, under ether anesthesia, with a solution of 2% paraformaldehyde, 0.1% glutaraldehyde in 0.12 M phosphate buffer (pH 7.2) for 20 min. After dissection, the brains were postfixed by immersion in the same fixative for 4 hr. The 1-d-old (P1) and P8 mouse pups resulting from the breeding of heterozygous *pcd* mice, as well as those transplanted with E12 C57BL cerebellar tissue, were perfused with a solution of 4% paraformaldehyde in the phosphate buffer for over 30 min. The dissected brains were postfixed in the same fixative by overnight immersion at 4°C. Blocks containing the cerebellum and attached brainstem of all fixed mice were prepared and infiltrated for 2 d in 30% sucrose.

β-Galactosidase detection. The appropriate cerebella were cut on a freezing microtome at 26 μm in either the sagittal or the frontal plane. β-Galactosidase activity was detected by incubating free-floating sections in a solution containing 2 mM $MgCl_2$, 4 mM $K_3Fe(CN)_6$, 4 mM $K_4Fe(CN)_6$, and 4 mg·ml^{-1} of 5-bromo-4-chloro-3-indoyl-β-D-galactopyranoside (X-gal; Bethesda Research Labs Life Technologies). The incubation was carried out at 37°C for 4 hr. After β-galactosidase detection, the sections were either double labeled with an anti-calbindin (CaBP) antibody or counterstained with neutral red.

Antibodies. The polyclonal anti-CaBP antiserum, obtained in rabbit against chick CaBP (Spencer et al., 1976), was kindly supplied by Dr. D. E. M. Lawson (Section of Neuroanatomy, Yale University). Rat-401 is a mouse monoclonal antibody (mAb) obtained from fixed spinal cord of E15 rat embryos (Hockfield and McKay, 1985), which recognizes nestin (Lendahl et al., 1990). The mAb Rat-401 was generously provided by Dr. Susan Hockfield (Institute of Animal Physiology and Genetics Research, New Haven, CT). These antibodies were used at dilutions of 1:8000 to 1:10,000 for anti-CaBP and 1:500 of the full-strength supernatant for mAb Rat-401.

Immunocytochemistry. For the cellular analysis of the Krox-20/lacZ14 cerebellum, the X-gal–stained sections were incubated overnight at room temperature with the polyclonal rabbit anti-CaBP antiserum, and processed according to the peroxidase–antiperoxidase (PAP) method of Sternberger et al. (1970). For study of the *pcd* cerebella grafted with the E12 transgenic cerebellar primordium, after the histochemical detection of β-galactosidase activity, the sections were also treated with the anti-CaBP antiserum. CaBP immunoreactivity was revealed in alternate sections either by the PAP method or by the immunofluorescence. The study of Rat-401 expression in postnatal and lesioned *pcd* cerebella was performed in free-floating frozen sections immunoprocessed with the mAb Rat-401 and revealed by the PAP method. Finally, the correlation between radial migration of grafted PCs and reexpression of glial Rat-401 antigen was analyzed in double-labeled sections. They were incubated overnight at room temperature with the mAb Rat-401 and the polyclonal rabbit anti-CaBP antibody. Rat-401 was revealed with the PAP method (Sternberger et al., 1970) and, thereafter, CaBP-immunoreactivity was visualized with a goat anti-mouse antibody conjugated to fluorescein isothiocyanate. Sections in which the incubation with primary antibodies was omitted were used as controls.

Results

Validation of the markers

β-Galactosidase activity identifies epithelial cells and Bergmann fibers from transgenic graft donors. Transgenic mouse lines carrying the Krox-20/lacZ hybrid gene have been generated. In one particular line, number 14, the hybrid gene is fully expressed in the CNS at P22. The gene product, as revealed by X-gal staining, is most abundant in the cerebellum, where it is present throughout all vermian (Fig. 1*A*) and hemispheric cortices, but only in the molecular layer. The staining covers the entire molecular layer (Fig. 1*A,B*), but is most intense at the interface between the molecular and granule cell layers, which appears as a fine scalloped band composed of alternating, irregular intensely pos-

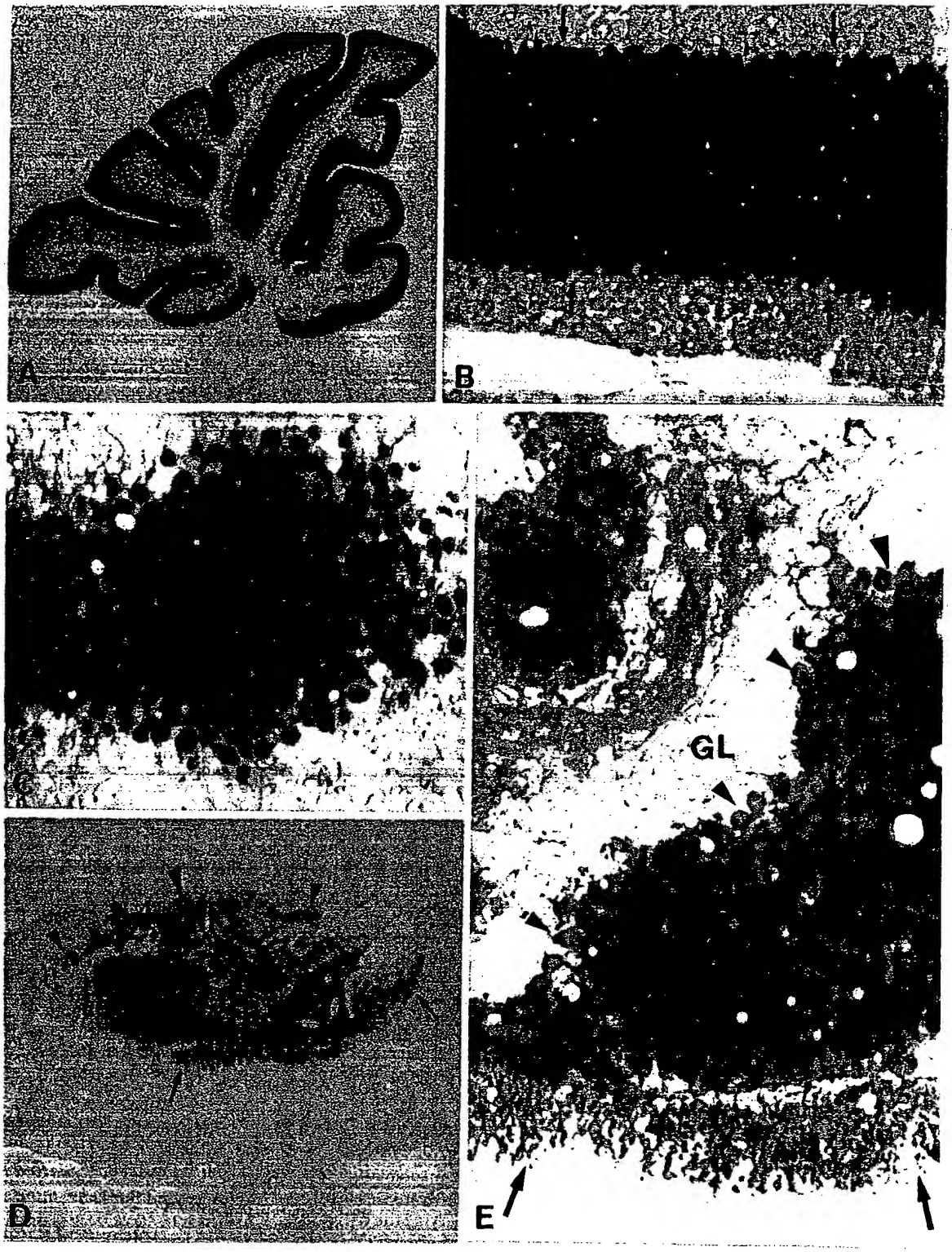

Figure 1. Expression of the Krox-20/lacZ transgene in P22 cerebellum of the transgenic mouse line (*A–C*), and 1 month after transplantation into adult *pcd* cerebellum (*D, E*). *A*, Blue X-gal–positive reaction is present all over the molecular layer of this midsagittal section, from lobule I to X. *B*, At higher magnification, the striate appearance of the blue staining is noticeable. Moreover, at the interface between molecular and granular layers, the blue reaction is stronger and exhibits a scalloped appearance (*arrows*). The section was lightly stained with neutral red to better reveal the cytoarchitectonic of the cerebellum. *C*, Double labeling with X-gal and CaBP of a transgenic mouse cerebellum cut in the frontal plane. The disposition of the cellular elements with blue, X-gal–positive reaction, surrounding the PC bodies—visualized by their CaBP immunoreactivity—permits their identification as Golgi epithelial cells. *D*, Double labeling, X-gal and CaBP, of a frontal section of a *pcd* cerebellum containing a large graft remnant (*GR*) in lobule VI, and numerous grafted PCs within the molecular layer of host lobules VI (*arrowheads*) and VIII (*arrows*). *E*, Higher magnification of the same graft as in *D* illustrating the presence of lacZ-expressing cells (blue reaction) in the molecular layer of two folia of the

Figure 2. Expression of Rat-401 antigen in postnatal, adult normal, and adult postlesioned *pcd* cerebellum. *A*, At P8, the mAb Rat-401 immunostains Bergmann fibers forming the characteristic palisades in the developing molecular layer. Note the lack of immunoreactive cells, other than Bergmann fibers, in the external granular layer (*EGL*). *B*, Four-month-old *pcd* cerebellum. The immunostaining identifies numerous cellular profiles exhibiting typical features of microglial cells, and dispersed throughout the molecular layer (*ML*). The *arrows* point to the interfolial surface. *C*, Three-month-old *pcd* cerebellum, 7 d after its posteroanterior perforation with the glass micropipette used for transplantation. Rat-401 reexpression occurs all along the cannula track in reactive astrocytes. The *arrows* point to molecular layer regions intercrossed by the lesion in which Bergmann fibers lack immunostaining, and the only reactive elements are microglial cells. Magnification: *A*, 340×; *B*, 275×; *C*, 130×.

itive and negative spots (Fig. 1*B*). Within the molecular layer, fine fibers, passing vertically through the cortex to the subpial surface, stand out against the blue background, giving this layer a radially striated appearance (Fig. 1*B*). On frontal sections passing tangentially through the PC layer (Fig. 1*C*), PC somata, identified by CaBP immunohistochemistry, occupy the unstained holes in the intensely blue sieve-like band at the molecular/granule cell layer interface. The only cellular elements in the cerebellum that correspond to those expressing β-galactosidase activity are the Golgi epithelial cells and their Bergmann fibers. This pattern of expression of the transgene in mouse line 14 likely results from its particular site of integration since it was not observed with the other lines (data not shown) and it does not correspond to the normal Krox-20 expression pattern (P. Topilko, S. Schneider-Maunoury, G. Levi, C. Babinet, and P. Charnay, unpublished observations).

mAb Rat-401 permits identification of migration-related Bergmann fibers in pcd *mutant mice.* In order to determine whether the *pcd* mutation affects postnatal expression of nestin, cerebella from the progeny of heterozygous mutant mice were immu-

graft remnant. The *arrowheads* point to grafted PC bodies in the graft remnant separating the molecular from the granular cell layer (*GL*). Note the lack of β-galactosidase reactivity in the host molecular layer containing grafted PCs (*arrows*). Magnification: *A*, 20×; *B*, 135×; *C*, 150×; *D*, 19×; *E*, 190×.

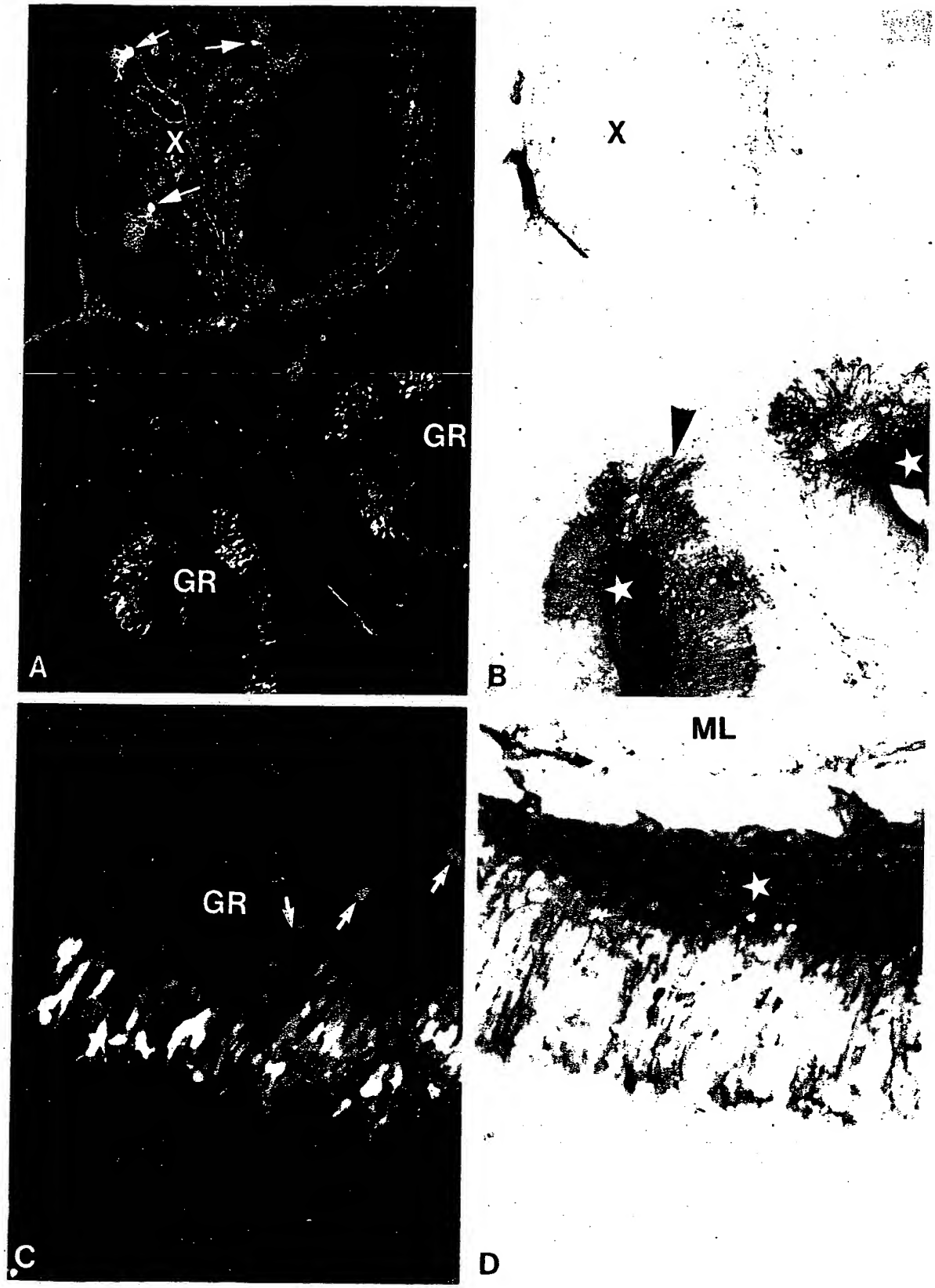

Figure 3. Rat-401 antigen expression in Bergmann fibers of adult *pcd* mice at 7 DAG. The mutant cerebella were sectioned sagittally, and double-labeled with mAb Rat-401 and anti-CaBP antisera. *A* and *B*, Low magnification of the same section (CaBP staining in *A* and Rat-401 in *B*). In lobule X (*X*) three *pcd* PCs remain (*arrows*), without preservation of Rat-401 in adjacent Bergmann fibers. The graft remnants (*GR*), located in the anterior lobe, exhibit an intense Rat-401 immunoreactivity (*stars*). Note the spatial correlation between the radial migration of CaBP-positive PCs

stained with mAb Rat-401 at P1 and P8, one litter at each ne period. This procedure was necessary since the clinical *pcd* enotype can be detected only after the second postnatal week, hereas granule cell migration along Bergmann fibers begins at rth and peaks at P8. Normal, heterozygous or homozygous ips could not be distinguished on the basis of the pattern of mmunostaining, at either P1 or P8, indicating that the *pcd* utation does not affect postnatal expression of nestin in the rebellum.

As in the rat (Hockfield and McKay, 1985), in addition to ergmann fibers that were already intensely immunopositive in e newborn mouse, but that are even clearer at P8 when charteristic palisades extending through the molecular and exterl granule cell layers were visible (Fig. 2*A*), cerebellar endoelial cells and astrocytic processes in the granule cell layer re labeled by the Rat-401 antibody. Diffuse but dense imunostaining could also be observed in white matter in both 1 and P8 mouse pups.

In cerebella of 2–4-month-old adult *pcd* mice, Bergmann fiers, astrocytic processes, and white matter have lost the Rat-01 antigen. Light staining remains visible, however, throughut the entire cortex, but most markedly in the atrophic molecular yer. In regions where this staining was most intense, cell bodies nd a network of highly ramified and crenelated processes, corsponding to microglial cells, could be observed (Fig. 2*B*). Also, s in lesion-induced reactive gliosis in the rat (S. Hockfield, rsonal communication), some astrocytes in the adult *pcd* cerbellum reexpressed the Rat-401 antigen. Figure 2*C* shows the sion made by the glass cannula used for implantation of the aft, the track of which is delineated by nestin-positive astroytic processes. Bergmann fibers adjacent to the lesion remained gative, however.

Detection of β-galactosidase in Bergmann fibers of pcd *mice rafted with Krox-20/lacZ14 transgenic cerebellar primordia*

ost mice, with grafts either in the cerebellar parenchyma or tween adjacent folia, were killed 1 or 2 months after grafting, hen grafted Bergmann fibers had reached maturity and fully xpressed the lacZ reporter gene. The same results were obtained gardless of the location of the grafts.

Figure 1*D* shows a large graft remnant within lobule VI, consting of a lobular, trilayered mini-cerebellar structure with aBP-positive PC dendrites in the molecular layer that is borered by the immunopositive PC cell bodies. PCs that had igrated into the host molecular layer (ventral molecular layer f lobule VI, dorsal molecular layer of lobule VIII) are visible ore peripherally (Fig. 1*D,E*). Expression of the lacZ gene in e graft remnant resembled that observed in the cerebellum of e transgenic mice. No β-galactosidase activity could be dected, however, in the molecular layer of the *pcd* host containing rafted PCs that have left the implant (Fig. 1*D,E*).

Golgi epithelial cells and their Bergmann fibers in the graft mnant, when they had matured, expressed the transgene with e same pattern as in P22 transgenic animals. These cells remained in the graft and did not migrate into the host molecular layer along with the PCs.

Spatiotemporal pattern of nestin (Rat-401 antigen) expression in Bergmann fibers of pcd *mice grafted with isogenic cerebellar primordia*

During tangential migration (DAG 5). In the animal analyzed, a small transplant was located partially within and partially outside of the cerebellum. In two of the folia adjacent to the intracerebellar portion of the graft, a few migrating PCs (horizontally oriented, bipolar, CaBP-positive cells) had invaded the surface of the host molecular layer. Immunostaining of the same sections with the Rat-401 antibody detected the presence of the antigen only in microglial cells which were lightly stained. No Bergmann fibers were labeled. The graft remnant was stained intensely by the Rat-401 antibody (data not shown).

During radial migration (DAG 7). Graft remnants, frequently present in the interfolial space between host molecular layers into which grafted PCs had migrated, contained only a few immature PCs (CaBP-positive neurons, almost devoid of processes) (Figs. 3*C*, 4*A*), but still exhibited diffuse and intense Rat-401 staining (Figs. 3*B,D*; 4*B*).

Migrating PCs (radially oriented, bipolar, CaBP-positive cells with short ascending and long descending processes, the latter frequently branched into two thinner processes) were visible at various depths and densities in the host molecular layer (Fig. 3*A,C*), which now expressed Rat-401 immunoreactivity. The antigen was expressed, however, only in areas with radially migrating PCs (Fig. 3*B,D*). In most instances, the density of migrating PCs was directly related to the intensity of Rat-401 staining of Bergmann fibers (Fig. 3). In some cases, however, the presence of a few migrating PCs had sufficed to induce intense antigen expression in the corresponding fibers. Narrow bands of immunoreactive fibers free of migrating PCs could also be observed (Fig. 3*A,B*). An extreme case of disparity between PC density and nestin expression is shown in Figure 4, *A* and *B*. In spite of these exceptions, the spatial correlation between reexpression of the Rat-401 antigen in host Bergmann fibers and the radial migration of grafted embryonic PCs is clear.

Postmigration (DAG 13). The PCs had finished their migration and were developing their mature dendritic trees. The CaBP-positive cells were either stellate or had multiple primary dendrites (Fig. 4*C*), characteristic of the transition from the "phase of stellate cells with disoriented dendrons" to the "phase of orientation and flattening of dendrites" (Ramon y Cajal, 1911).

Bergmann fibers in the host no longer express the Rat-401 antigen. Only lightly stained microglial cells could be detected in the molecular layer (Fig. 4*D*).

Discussion

When embryonic PCs migrate from an implant into the molecular layer of adult PC-deficient hosts, the final stage of migration is guided by Bergmann glial fibers (Sotelo and Alvarado-Mallart, 1987b; Sotelo et al., 1990). The question asked in the

ithin the host molecular layer, and the reexpression of Rat-401 antigen by host Bergmann fibers. The *arrowhead* points to a small region of host olecular layer with reexpression of Rat-401 staining but without apparent migrating PCs. *C* and *D*, Higher magnification of another transplanted rebellum. The graft remnant (*GR*) is almost devoid of CaBP-positive neurons (*arrows*) but is intensively stained by Rat-401 antibody (*star*). umerous CaBP-positive neurons migrate radially in the host molecular layer, a region where host Bergmann fibers express Rat-401 antigenicity. ote, in the adjacent folium, that the molecular layer (*ML*) is devoid of migrating PCs and of Rat-401–immunolabeled Bergmann fibers. Magnitation: *A* and *B*, 75×; *C* and *D*, 270×.

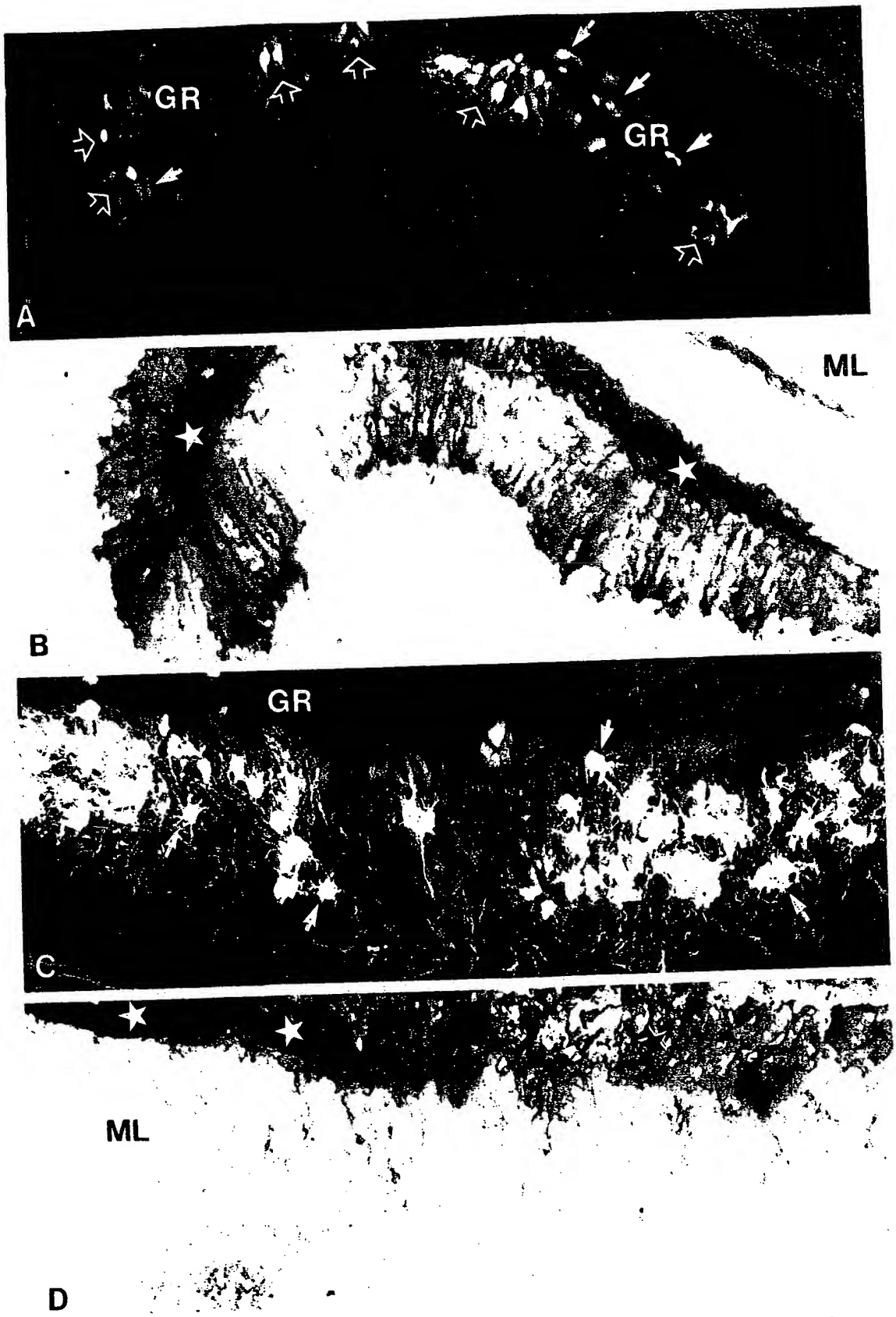

Figure 4. Rat-401 immunostaining of host Bergmann fibers at 7 DAG (*A, B*) and its extinction at 13 DAG (*C, D*): sagittally cut cerebella, double labeled with mAb Rat-401 and anti-CaBP antisera. *A* and *B*, At 7 DAG, the graft remnant (*GR*) is intensely immunostained by Rat-401 (*stars*) and contains a few CaBP-positive neurons (*solid arrows*). The host molecular layer adjacent to the graft remnant contains Rat-401–immunoreactive

present study was whether these were adult fibers from the host, and if so, how did they accomplish this task, or whether embryonic glial cells have also comigrated and thus express the appropriate molecular signals to facilitate PC invasion.

Radial glial and Bergmann fiber as neuronal migratory axes

The concept of glial guidance, derived from the observation of a tight association between migrating granule cells and Bergmann fibers in the developing molecular layer of the cerebellum (Rakic, 1971), underlies a commonly used mechanism for the positioning of young postmitotic neurons in developing brain. PC migration, during development, is guided, if at all, by radial glia not Bergmann fibers, which appear too late in fetal development and do not span the necessary distance from the ventricular neuroepithelium to the nascent PC plate (Misson et al., 1988). Bergmann fibers do, however, guide the migration of granule cells postnatally (Rakic, 1971).

Host and not donor Bergmann fibers subserve migration to grafted PCs

Bergmann fibers are specialized astrocytes (Ramon y Cajal, 1911), which can develop from radial glial cells (Rakic, 1984), and may therefore share the phenotypic traits that allow both to guide the migrati n of a given type of neuron. Previous studies have shown that implanted glial cells (oligodendrocytes and astrocytes) are indeed able to migrate into host brains. Although most of the experiments involved grafts into newborn hosts (Gumpel et al., 1983; Jacque et al., 1986, 1992; Suard et al., 1989; Zhou et al., 1990), migration of grafted astrocytes has also been observed in adult brain (Lindsay and Raisman, 1984; Goldberg and Bernstein, 1988). In grafting experiments like those presented here, vimentin-positive astrocytic processes that straddle the graft and the *pcd* host molecular layer have been observed by DAG 6 (Sotelo et al., 1990).

The present study, in which Bergmann fibers developing in an embryonic implant would specifically express the reporter lacZ gene (M. Frain, P. Charnay, R. Werhle, and C. Sotelo, unpublished observations), demonstrated that β-galactosidase–positive fibers remain in the graft. First of all, no blue-stained fibers were observed in the host molecular layer. Second, the intensity of staining in the graft remnants suggests that there has been no loss of lacZ-expressing cells from the graft. Nevertheless, one cannot exclude that there has been proliferation of β-galactosidase–positive cells in the graft or after migration, and that these cells in their new environment, if they acquire the Bergmann phenotype, no longer express the transgene. Previous studies with tritiated thymidine have revealed, however, that the only autoradiographically labelled nuclei in the transplanted *pcd* cerebellum belong to grafted PCs (Sotelo et al., 1990). Therefore, it is highly probable that adult host Bergmann fibers guide the migration of grafted PCs.

This "abnormal" migration strongly suggests that a common mechanism must exist in cerebellum for guiding neurons to their appropriate position, regardless of the type of neuron or the glial cell involved. This may be an illustration *in vivo* of the "passive generic" guidance evoked by Hatten (1990) to explain the *in vitro* migration of hippocampal or cerebellar neurons on glia from the heterologous region. Glial cells that present migratory axes, whatever their age, differentiated state, or region of origin, must therefore be capable of expressing the molecules necessary for the neuron/glial interaction to take place.

Transient Rat-401 expression in glial axes subserving neuronal migration

What are the molecules involved in the neuron/glial interaction? A large number of studies have attempted to elucidate the molecular mechanisms of glial guidance (Lindner et al., 1983, 1986; Persohn and Schachner, 1987). Multiple receptor systems are thought to be involved (Tomaselli et al., 1988; Hatten, 1990); for example, surface adhesion molecules on neurons and glia (Lindner et al., 1983) and cytoskeleton-related receptors such as the integrins (Hynes, 1987). The present study suggests that one of these molecules may be the intermediate filament nestin (Lendahl et al., 1990), recognized by mAb Rat-401, that was expressed only in those Bergmann fibers on which grafted PCs were migrating, and in the same time window that has been reported for radial migration of these grafted neurons (Sotelo et al., 1990).

The regulatory factors governing nestin expression have not been determined, but its timing and localization have been described. Present in almost all cells in the proliferative zone of the rat neural tube before neurons differentiate, it has been considered to be a marker of the immediate precursor to neurons (Lendahl et al., 1990). Nevertheless, nestin is also present in glial cells. Hence, coexpression of the Rat-401 antigen and glial fibrillary acidic protein has been observed in primary cultures, presumably in glial cells (Frederiksen and McKay, 1988). The anti-nestin antibody also stains radial glial cells during development (Hockfield and McKay, 1985), which may mean that these cells not only play an important role in neuronal migration, but may also be neuronal precursors (Lendahl et al., 1990). In addition to its ostensible role in neuronal differentiation and guidance, expression of low levels of nestin in microglia and reexpression in reactive astrocytes suggests that it has other functions as well. The nature of these functions is unknown.

In the developing rat cerebellum, the nestin gene is expressed from E19 to P9 with a peak at P5 (Lendahl et al., 1990). This does not coincide with proliferation of neurons from the ventricular epithelium (E12–E16). Neurons in the external granule layer are born between P1 and P19 (Altman, 1982), but studies in rat (Hockfield and McKay, 1985) and mouse (present results) suggest that none or only a few precursor cells in this secondary neuroepithelium may express the nestin protein, and ultrastructural analysis would be needed to determine the nature of these cells. The transient expression of the nestin gene (Lendahl et al., 1990) and protein (Hockfield and McKay, 1985; present results)

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Bergmann fibers. In this exceptional situation, this region of molecular layer has very few grafted PCs (*open arrows*), in comparison to the intensity and extent of Rat-401–positive Bergmann fibers. *ML* marks the molecular layer of the adjacent folium, free of migrating PCs and of Rat-401–stained Bergmann fibers. *C* and *D*, At 13 DAG the graft remnant (*GR*) is always immunostained by the mAb Rat-401 (*stars*), but the adjacent host molecular layer (*ML*) has missed its Rat-401–positive Bergmann fibers (only the light microglial staining is visible). The disappearance of Bergmann fibers' immunoreactivity coincides with the arrest of grafted PC migration. These neurons are now dispersed throughout the host molecular layer, and are building up their dendritic arbors (most of these PCs are in the phase of "stellate cells with disoriented dendrons"; *arrows*). Magnification, 220× for *A–D*.

is, however, correlated with the appearance of Golgi epithelial cells and their Bergmann fibers (Miale and Sidman, 1961; Shiga et al., 1983). Nestin, in postnatal cerebellum, may therefore be a marker, not of neuronal proliferation, but of Bergmann fibers during granule cell migration.

Migration of grafted PCs and transient reexpression of Rat-401 in host Bergmann fibers

The present study suggests that nestin is also a marker of Bergmann fibers guiding the ectopic and heterochronic migration of grafted PCs in the adult host. Indeed, the expression of nestin in these adult glial fibers seems to be regulated by the presence of the grafted PCs, given the spatiotemporal correlation between radial migration and antigen expression reported here. This is not the only example of a PC-related change in Bergmann fiber phenotype. Late degeneration of PCs in the cerebella of *nervous*, *pcd*, and *lurcher* mutant mice (Fisher, 1984), and in murine chimeras (Fisher and Mullen, 1988), has been reported to be temporally correlated with loss of a specific adult isozyme of *sn*-glycerol-3-phosphate dehydrogenase (GPDH) that is normally present in the fibers. The mechanism underlying this phenomenon would appear to be different, however, from the induction of nestin expression observed in the present study. Whereas GPDH expression seems to require sustained interaction between the neurons and the glia, the presence of normal PCs in the adult cerebellum and the nodulus of the *pcd* cerebellum does not prevent the developmentally regulated disappearance of the Rat-401 antigen from Bergmann fibers, nor does the proximity of grafted PCs, at least at DAG 13, sustain reexpression of the antigen beyond the period of radial migration. It seems probable that a surface antigen and/or a soluble molecule of limited diffusion, expressed by PC only during migration, regulates the level and duration of nestin expression in the Bergmann fibers, but the nature of the signaling molecules remains to be determined.

In conclusion, this study has shown that adult glial cells can change their phenotypic expression in the presence of embryonic neurons, recapitulating transient phenotypes associated with specific stages of normal development. In the sense that grafted embryonic PCs seem to regulate gene expression in neural cells of the adult host, we have termed this new type of glial transformation "adaptive rejuvenation." These changes generate, transiently, a microenvironment permitting radial migration of the PCs, the necessary precondition for their synaptic integration and the restoration of normal circuitry in the cerebellar cortex of *pcd* mice (Sotelo and Alvarado-Mallart, 1987b).

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